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In-vitro analysis of physical and chemical parameters of rice straw to produce high amount of reducing sugars

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ABSTRACT

Agro wastes are the most abundantly available source of lignocelluloses. Rice straw is one of the most abundant agricultural by-products throughout the world. It generally contains approximately 39% cellulose, 27% hemicellulose and 12% lignin. Cellulose and hemicelluloses present in rice straw can be degraded by the use of microorganisms, this enzymatic hydrolysis can be enhanced by chemical and physical techniques, which yield fermentable sugars. In this study, optimisation of the chemical and physical parameters for lingo cellulolysis on rice straw with *Bacillus pumilus* was investigated for obtaining maximum amount of reducing sugars which could be used for bioethanol production. The physical parameters were pH, Incubation Temperature, High Temperature and the chemical parameters were Acid, Ammonium nitrate and Maltose Concentration. The optimised conditions obtained for the microorganism and the substrate rice straw was at pH-4.0, Incubation Temperature-25ºC, High Temperature-200ºC, Acid-0.9%, Ammonium nitrate-0.5%, Maltose-2.0%.

Key Words: Bioethanol, *Bacillus pumilus*, Lignin, Hemicellulose, Reducing sugars.

1. INTRODUCTION

Microbial biodiversity is a daunting task. Exploration of microbial diversity is clearly a topic of considerable importance and interest. Besides, analysis of microbial biodiversity also helps in isolating and identifying new and potential microorganisms having high specificity for recalcitrant compounds Surajit Das et al. (2006). The renewable energy is vital as non-renewable energy becomes more scarce and expensive. The use of diverse biomass resources like agro and industrial waste is projected to contribute for future energy demands. Biomass is one of the most important raw materials in bio-ethanol production Balat et al. (2008). Agro waste or the plants containing complex sugar, cellulose, lignin and starch can be easily converted into simple sugars and fermented using microorganisms. This concept of bioethanol production has led to growing interests in alternative, non-edible biomass resources. Ligno cellulosic biomass, such as wood, straw and grasses, are viewed as important sources and optimisation of these substrate for the degradation of complex sugars may help in meeting the demand of bioethanol in the world. Ethanol is a renewable fuel that can be used as partial gasoline replacement. Demand for ethanol will increase with reduction in petroleum production. Production of fuel ethanol from lignocellulose materials such as rice straw is advantageous because of local availability. To take this advantage, it is important to convert the materials into fermentable sugars as much as possible Nakorn Tippayawong et al. (2011).

The physical and chemical Pre-treatment process are the most important step in conversion of cellulose to ethanol, as the treatment can remove hemicelluloses, reduce cellulose crystallinity and increase the porosity of materials. Pre-treatments improve the digestibility of the lignocellulosic materials. The physical parameters mainly are pH, Incubation temperature, High temperature where as chemical parameters are Acid concentration, Nitrogen and Carbon source with different concentrations.

2. MATERIALS AND METHODOLOGY

2.1. Isolation and Identification of the Microorganism

The microorganism was isolated from the back water sediment sample from Cuddalore district in India. The microorganism was identified by basic microbiological staining and biochemical tests and was identified as *Bacillus pumilus*. These were sub cultured and the pure cultures were used for preliminary identification for their property to degrade cellulose, Hemicellulose and Lignin. The microbe had the capacity to degrade all the three substrates. The isolate was thus utilised to breakdown rice straw, an abundantly available agro waste throughout the world. The rice straw was degraded by the isolate to release simple sugars and lignin oxidation was observed. The enhancement of this degradation was carried out using various physical and chemical parameters.

2.2. Optimisation of Physical Parameters

2.2.1. pH

The substrate-Rice straw were collected and 5g of the substrate was weighed separately ground into small pieces which was then transferred to different flasks containing 100 ml of water. Different pH 4, 5, 6, 7,8 and 9 were set using 1N NaOH and 1N HCl (Pezsa and Ailer 2011). The flasks were autoclaved at 121^{0}C for 15 minutes at 15psi. Once the flasks had cooled, they were inoculated with *Bacillus pumilus* for the formation of reducing sugars. Reading was taken for reducing sugar estimation using DNS (Miller, 1959) and lignin degradation assay using veratryl alcohol (Frederick, 1992). The readings were further taken weekly for 8 consecutive weeks.

2.2.2. Incubation Temperature

5 g of substrate was ground into small pieces and added into flasks each containing 100ml of water and sterilised at 121^{0}C for 15 minutes at 15 psi. Once the flasks had cooled, they were inoculated with *Bacillus pumilus* in sterile condition. After inoculation, the flasks were incubated at 4 different temperatures to study the impact of the incubation temperature on lingo cellulose degradation. The temperatures at which the incubation was done were 25, 30, 37 and 40°C Charitha Devi et al. (2012). Reading was taken for reducing sugar estimation using DNS (Miller 1959) and lignin degradation assay using veratryl alcohol (Frederick, 1992). The readings were further taken weekly for 8 consecutive weeks.

2.2.3. High Temperature

5 g of substrate was ground into small pieces and was added in flasks containing 100ml of water and kept at different temperatures i.e., 100^{0}C, 150^{0}C, 200^{0}C and 250^{0}C for 1hr (Brownell and Saddler, 1986). The lid of the flasks was kept unclosed in hot air oven. Once the flasks had cooled, they were inoculated with *Bacillus pumilus*. Reading was taken for reducing sugar estimation using DNS

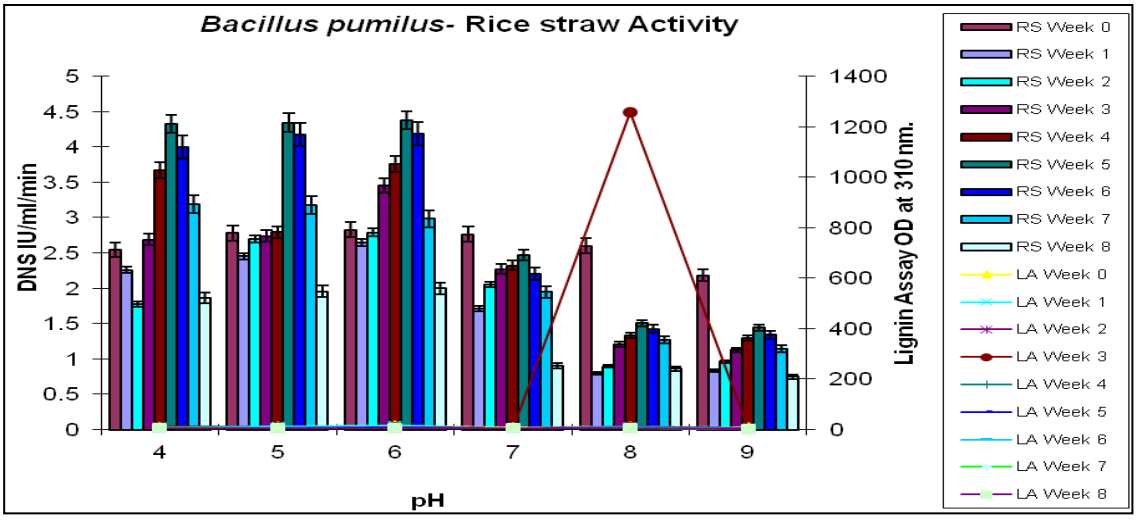

Figure 1

Optimisation of pH for lignocellulosic degradation by *Bacillus pumilus* on Rice straw

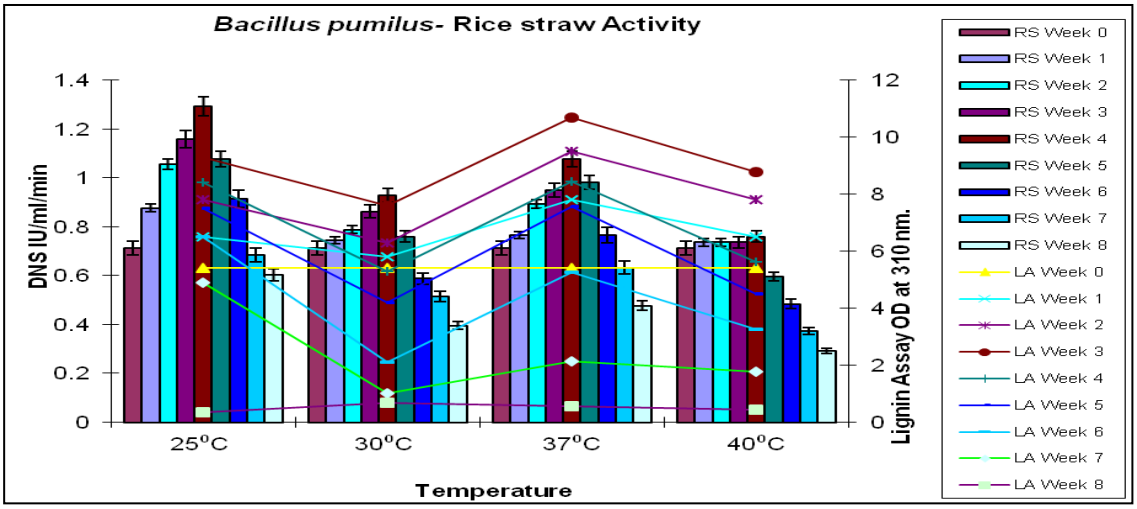

Figure 2

Optimisation of Incubation Temperature for lignocellulosic degradation by *Bacillus pumilus* on Rice straw

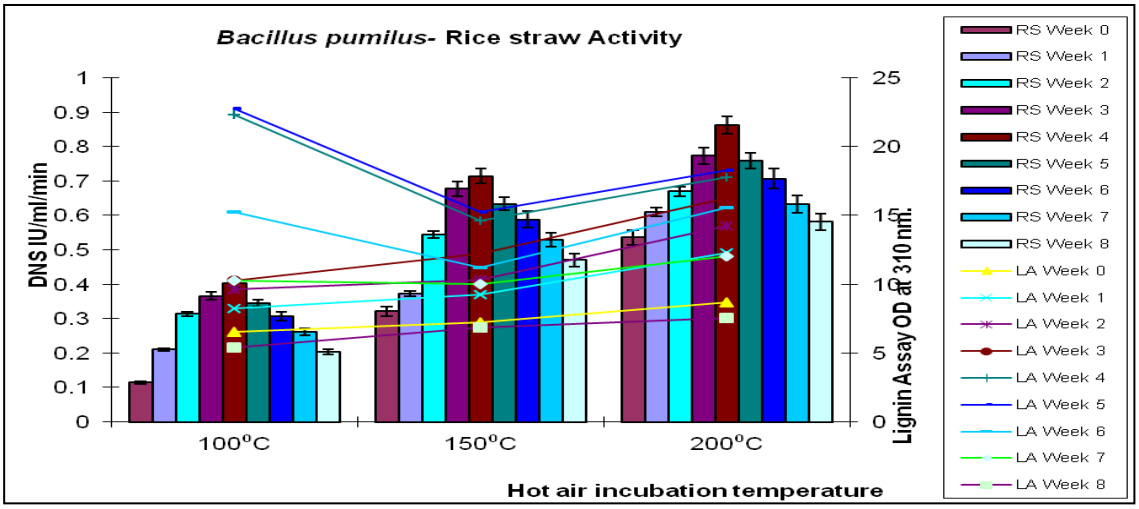

Figure 3

Optimisation of High Temperature for lignocellulosic degradation by *Bacillus pumilus* on Rice straw

(Miller, 1959) and lignin degradation assay using veratryl alcohol (Frederick, 1992). The readings were further taken weekly for 8 consecutive weeks.

2.3. Optimisation of Chemical Parameters

2.3.1. Acid

5 g of rice straw was ground into small pieces and then added into flasks containing 100ml of different concentrations of acid 0.1%, 0.3%, 0.5%, 0.7%, 0.9% and 1% (Leenakul and Tippayawong, 2010) and Nutawan et al. (2010). The flasks were left at room temperature for 24 hours. The substrates were then neutralized Umbrin et al. (2011) and autoclaved at 121^{0}C for 15 minutes at 15 psi. Once the flasks had cooled, they were inoculated with *Bacillus pumilus*. Reading was taken for reducing sugar estimation using DNS (Miller, 1959) and lignin degradation assay using veratryl alcohol (Frederick, 1992). The readings were further taken weekly for 8 consecutive weeks.

2.3.2. Nitrogen Source-Ammonium nitrate

Different concentrations of Ammonium nitrate (0.5%, 1.0%, 1.5% and 2.0%) were taken into flasks. 5 g of substrate was ground/cut into small pieces and was added with 100ml distilled water. The flasks were then sterilised at 121^{0}C for 15 minutes

at 15 psi. Once the flasks had cooled, they were inoculated with *Bacillus pumilus*. Reading was taken for reducing sugar estimation using DNS (Miller, 1959) and lignin degradation assay using veratryl alcohol (Frederick, 1992). The readings were further taken weekly for 8 consecutive weeks.

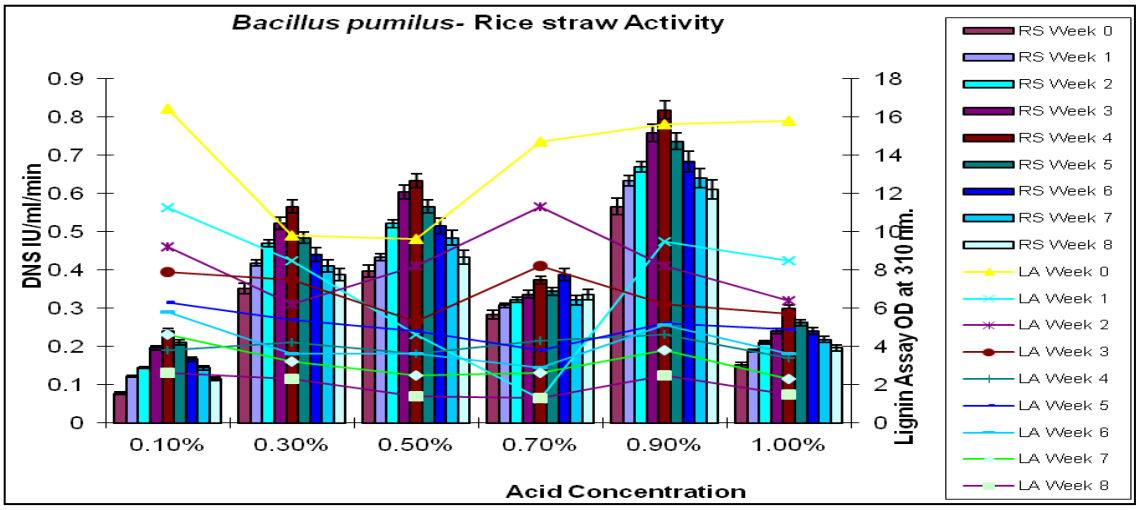

Figure 4

Optimisation of Acid concentration for lignocellulosic degradation by *Bacillus pumilus* on Rice straw

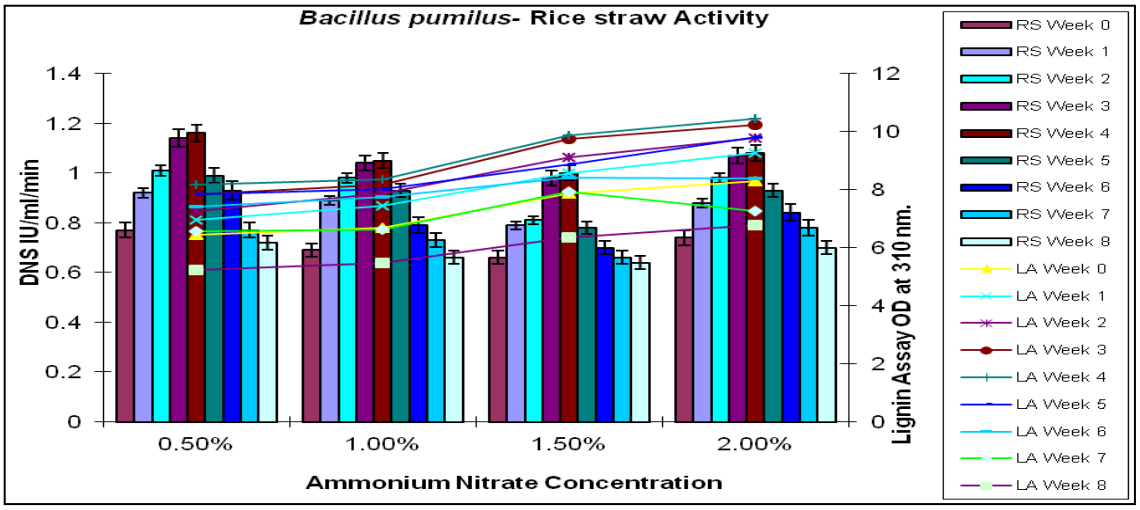

Figure 5

Optimisation of nitrogen source (Ammonium nitrate) for lignocellulosic degradation by *Bacillus pumilus* on Rice straw

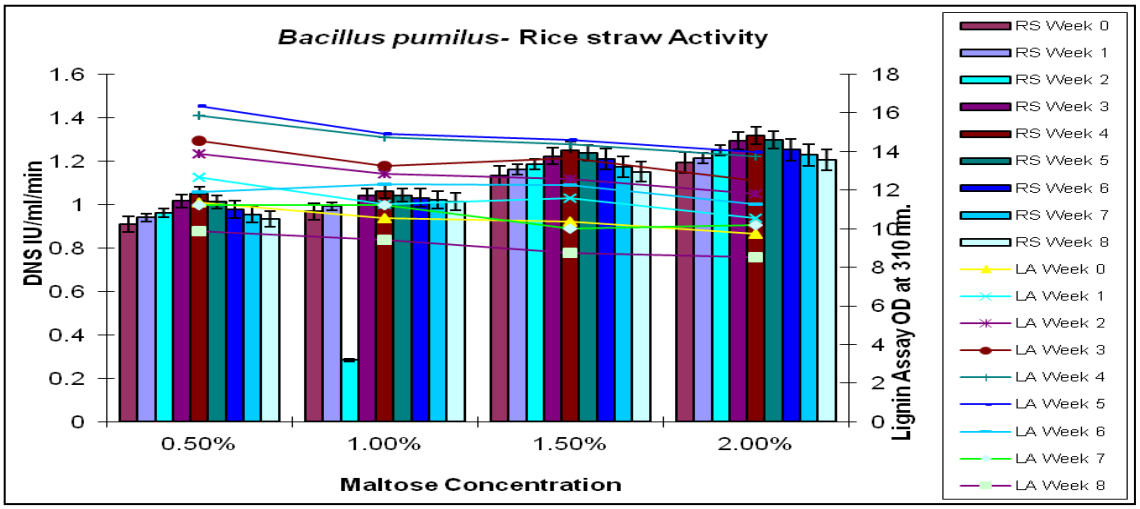

Figure 6

Optimisation of carbon source (Maltose) for lignocellulosic degradation by *Bacillus pumilus* on Rice straw

2.3.3 Carbon Source- Maltose

5 g of substrate was ground/cut into small pieces and was added into flasks containing 100ml water with different concentrations of carbon sources - 0.5%, 1.0%, 1.5% and 2.0% Mehdi Dashtban et al. (2011). The flasks were then autoclaved at 121^{0}C for 15 minutes at 15 psi. Once the flasks had cooled, they were inoculated with *Bacillus pumilus*. Reading was taken for reducing sugar estimation using DNS (Miller, 1959) and lignin degradation assay using veratryl alcohol (Frederick, 1992). The readings were further taken weekly for 8 consecutive weeks.

3. RESULTS AND DISCUSSION

The microorganisms were identified to be *Bacillus pumilus* by basic microbiological technique. The preliminary test indicated the property of the microorganism to degrade complex sugars to simple sugars by producing enzymes. The optimisation of the substrate and the microorganism places a major role in degradation of the substrate and in increasing the production of the enzymes as well as maintaining the viability of the microorganism. The physical parameters investigated in this study were pH (Figure 1), Incubation (Figure 2) and High Temperature (Figure 3). Parameters like pH and Incubation temperature has a vital role in the growth of the microorganism. The optimum pH for *Bacillus pumilus* with rice straw was found to be 4.0 and the organisms showed luxuriant growth at the incubation temperature of 25ºC similarly (Zayed and Meyer, 1996) examined *Trichoderma viridae* and *Aspergillus niger* for their ability to produce fermentable sugars from cellulosic waste like wheat straw at 25° to 30°C within 3 days. The release of reducing sugars was seen maximum when the substrate was incubated at 200ºC in the hot air oven where as Waksman et al. (1939) found that the highest degradation of lignin occurred at 50ºC during horse manure and straw compost.

The chemical parameters like acid hydrolysis of the substrate, supplementation of nitrogen and carbon source at various concentrations helps in the growth of the microorganism and maintaining the structural composition of the substrate. The chemical parameter where in the lignocelluloses degradation was maximum at 0.9% concentration of acid (Figure 4), similar strategy was carried out by Soderstroum et al., (2002a, b) where he investigated two stage pre-treatment of spruce with SO_2 or H_2SO_4, when usin SO_2, the optimal conditions were found to be impregnation with 3.0% SO_2 and the first pre-treatment step at 190^{0}C for 2 min and second pre-treatment step at 220^{0}C for 5min. 0.5% of the nitrogen source ammonium nitrate (Figure 5). Carbon source-maltose (Figure 6) did not show varied difference with the concentration and the maximum degradation was seen when the maltose concentration was 2.0% in the medium containing the substrate. In the present investigation the pre-treatment of rice straw showed an increase in the formation of simple sugars with the help of *Bacillus pumilus* as similar to the investigations carried out by Dale et al. (1999), Muthukumarappan et al. (2007) who showed a significant improvement on sugar recovery from corn strove, switch grass and Indian grass.

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